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ifw

In re application of: Sunil KOCHHAR et al.

Confirmation No.: 2216

Application No.: 10/691,590

Group Art Unit: 1761

Filing Date: October 24, 2003

Examiner:

For: COCOA POLYPEPTIDES AND THEIR USE
IN THE PRODUCTION OF COCOA AND
CHOCOLATE FLAVOR

Attorney Docket No.: 88265-6820

SUBMISSION OF PRIORITY DOCUMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

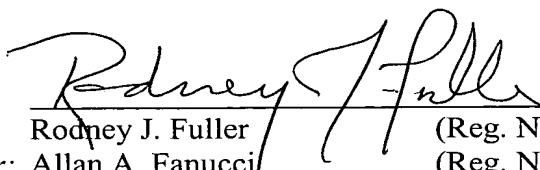
Applicants have claimed priority under 35 U.S.C. § 119 of European
Application No. 01110251.4 filed April 25, 2001. In support of this claim, a certified copy of
said application is submitted herewith.

No fee or certification is believed to be due for this submission. Should any
fees be required, however, please charge such fees to Winston & Strawn LLP Deposit
Account No. 50-1814.

Respectfully submitted,

6/4/04

Date



Rodney J. Fuller (Reg. No. 46,714)
For: Allan A. Fanucci (Reg. No. 30,256)

WINSTON & STRAWN
Customer No. 28765

202-371-5838



**Europäisches
Patentamt**

**European
Patent Office**

**Office européen
des brevets**

Bescheinigung

Certificate

Attestation

Die angehefteten Unterla-
gen stimmen mit den in
den Akten befindlichen
Unterlagen der unten be-
zeichneten europäischen
Patentanmeldung überein
(Regel 94(4) EPU).

The attached is a true copy
of documents contained in
the European patent appli-
cation indicated below
(Rule 94(4) EPC).

Les documents ci-annexés
sont conformes aux
documents figurant dans
le dossier de la demande
de brevet dont le numéro
est indiqué ci-dessous
(règle 94(4) CBE).

Patentanmeldung Nr. Patent application No. Demande de brevet n°

01110251.4

München, den
Munich,
Munich, le

16/10/03

Der Präsident des Europäischen Patentamts;
Im Auftrag.

For the President of the European Patent Office.

Le Président de l'Office européen des brevets
p.o.

W. Claessens



Antrag auf Erteilung eines europäischen Patents / Request for grant of a European patent / Requête en délivrance d'un brevet européen 1

Bestätigung einer bereits durch Telefax eingereichten Anmeldung / Confirmation of an application already filed by facsimile / Confirmation d'une demande déjà déposée par téléfax
Wenn ja, Datum der Übermittlung des Telefax und Name der Einreichungsbehörde / If yes, facsimile date and name of the authority with which the documents were filed / Si oui, date d'envoi du téléfax et nom de l'autorité de dépôt

☐ Ja / Yes / Oui

Datum / Date

Behörde / Authority / Autorité

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Anmeldenummer / Application No. / N° de la demande	MKEY	1	01110251.4
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	2	25.04.01
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	3	
Anmeldetag / Date of filing / Date de dépôt		4	

Tabulatoren-Positionen / Tabulation marks / Arrêts de tabulation

Es wird die Erteilung eines europäischen Patents und gemäß Artikel 94 die Prüfung der Anmeldung beantragt / Grant of a European patent, and examination of the application under Article 94, are hereby requested / Il est demandé la délivrance d'un brevet européen et, conformément à l'article 94, l'examen de la demande	EXAM 4	5	<input checked="" type="checkbox"/> Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt II, 5): / Request for examination in an admissible non-EPO language (see Notes II, 5): / Requête en examen dans une langue non officielle autorisée (voir notice II, 5): Si richiede di esaminare la domanda ai sensi dell'art. 94.
Zeichen des Anmelders oder Vertreters (max. 15 Positionen) / Applicant's or representative's reference (maximum 15 spaces) / Référence du demandeur ou du mandataire (max. 15 caractères ou espaces)	AREF	6	E 80273 EP
Anmelder / Applicant / Demandeur Name / Nom		7	Société des Produits Nestlé S. A.
Anschrift / Address / Adresse		8	P. O. Box 353
			1088 Vevey
			Switzerland
	APPR 01 # 2292282		
# DEST #			
Zustellanschrift / Address for correspondence / Adresse pour la correspondance		9	
	PADR		
Staat des Wohnsitzes oder Sitzes / State of residence or of principal place of business / Etat du domicile ou du siège		10	CH
Staatsangehörigkeit / Nationality / Nationalité		11	CH
Telefon / Telephone / Téléphone		12	
Telex / Téllex	Telefax / Fax / Téléfax	13	
Weitere(r) Anmelder auf Zusatzblatt / Additional applicant(s) on additional sheet / Autre(s) demandeur(s) sur feuille additionnelle		14	
Vertreter / Representative / Mandataire Name / Nom		15	Becker, Kurig, Straus Zusammenschluß 157
(Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen ist und an den zugestellt wird / Name only one representative who is to be listed in the Register of European Patents and to whom notification is to be made / N'indiquer qu'un seul mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)			
	FREP 01 10157A8#		
Geschäftsanschrift / Address of place of business / Adresse professionnelle		16	Becker, Kurig, Straus Bavariastr. 7 80336 München
Telefon / Telephone / Téléphone		17	089-746 303-0
Telex / Téllex	Telefax / Fax / Téléfax	18	089-746 303-11
Weitere(r) Vertreter auf Zusatzblatt / Additional representative(s) on additional sheet / Autre(s) mandataire(s) sur feuille additionnelle		19	


Vollmacht / Authorisation / Pouvoir ist beigelegt / is enclosed / joint ist registriert unter Nummer / has been registered under No. / a été enregistré sous le n°	20	<input type="checkbox"/>																
GENA	21	<input type="checkbox"/>	Nummer Number Numéro															
Erfinder / Inventor / Inventeur Anmelder ist (sind) alleinige(r) Erfinder / The applicant(s) is (are) the sole inventor(s) / Le(s) demandeur(s) est (sont) le (les) seul(s) inventeur(s) Erfindernennung in gesondertem Schriftstück / Designation of inventor attached / Voir la désignation de l'inventeur ci-jointe	22	<input type="checkbox"/>																
INVT 20 # #	23	<input type="checkbox"/>																
Bezeichnung der Erfindung / Title of invention / Titre de l'invention <div style="display: flex; justify-content: space-around; border: 1px solid black; padding: 2px;"> TIDE TIEN TIFR </div>	24	<input type="checkbox"/>	Cocoa polypeptides and their use in the production of cocoa and chocolate flavour															
Prioritätserklärung / Declaration of priority / Déclaration de priorité	25	<input type="checkbox"/>	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 33%;">Staat / State / Etat</th> <th style="width: 33%;">Anmeldetag / Date of filing / Date de dépôt</th> <th style="width: 33%;">Aktenzeichen / Application No. / N° de la demande</th> </tr> <tr><td>1</td><td></td><td></td></tr> <tr><td>2</td><td></td><td></td></tr> <tr><td>3</td><td></td><td></td></tr> <tr><td>4</td><td></td><td></td></tr> </table>	Staat / State / Etat	Anmeldetag / Date of filing / Date de dépôt	Aktenzeichen / Application No. / N° de la demande	1			2			3			4		
Staat / State / Etat	Anmeldetag / Date of filing / Date de dépôt	Aktenzeichen / Application No. / N° de la demande																
1																		
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3																		
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01 # . . . # . . . # . . . 02 # . . . # . . . # . . . 03 # . . . # . . . # . . . 04 # . . . # . . . # . . . Weitere Prioritätserklärung(en) auf Zusatzblatt / Additional declaration(s) of priority on additional sheet / Autre(s) déclaration(s) de priorité sur feuille additionnelle	25a	<input type="checkbox"/>																
Es wird hiermit erklärt, daß die Anmeldung eine vollständige Übersetzung der früheren Anmeldung ist (Regel 38(4)) / It is hereby declared that the application is a complete translation of the previous application (Rule 38(4)) / Il est déclaré par la présente que la demande est une traduction intégrale de la demande antérieure (règle 38(4))	26	<input type="checkbox"/>	Matière biologique L'invention concerne et/ou utilise de la matière biologique, déposée conformément à la règle 28.															
Biologisches Material Die Erfindung bezieht sich auf bzw. verwendet biologisches Material, das nach Regel 28 hinterlegt worden ist. Die Angaben nach Regel 28(1)(c) (falls noch nicht bekannt, die Hinterlegungs- stelle und das (die) Bezugs- zeichen (Nummer, Symbole usw.) des Hinterlegers) sind in den technischen Anmeldungsunterlagen enthalten auf / The particulars referred to in Rule 28(1)(c) (if not yet known, the depository institution and the identification reference(s) [number, symbols etc.] of the depositor) are given in the technical documents in the application on / Les indications visées à la règle 28(1)(c) (si pas encore connues, l'autorité de dépôt et la (les) référence(s) d'identification [numéro ou symboles etc.] du déposant) figurent dans les pièces techniques de la demande à la /aux	27	<input type="checkbox"/>	<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 50%;">Seite(n) / page(s)</th> <th style="width: 50%;">Zeile(n) / line(s) / ligne(s)</th> </tr> </table>	Seite(n) / page(s)	Zeile(n) / line(s) / ligne(s)													
Seite(n) / page(s)	Zeile(n) / line(s) / ligne(s)																	
Biological material The invention relates to and/or uses biological material deposited under Rule 28. BIOM 1 # #	27a	<input type="checkbox"/>																
werden später mitgeteilt / will be submitted later /seront communiquées ultérieurement Die Empfangsbescheinigung(en) der Hinterlegungsstelle ist (sind) beigelegt / The receipt(s) of deposit issued by the depository institution is (are) enclosed / Le(s) récépissé(s) de dépôt délivré(s) par l'autorité de dépôt est (sont) joint(s)	27b	<input type="checkbox"/>																
wird (werden) nachgereicht / will be filed later /sera (seront) produit(s) ultérieurement	27c	<input type="checkbox"/>																

<p>Falls das biologische Material nicht vom Anmelder, sondern von einem Dritten hinterlegt wurde: / Where the biological material has been deposited by a person other than the applicant: / Lorsque la matière biologique a été déposée par une personne autre que le demandeur:</p> <p>Ermächtigung nach Regel 28(1)(d) / Authorisation under Rule 28(1)(d) / L'autorisation en vertu de la règle 28(1)(d)</p> <p>ist beigefügt / is enclosed / est jointe</p> <p>wird nachgereicht / will be filed later / sera produite ultérieurement</p>	<p>28</p> <p>28a <input type="checkbox"/></p> <p>28b <input type="checkbox"/></p>	<p>Name und Anschrift des Hinterlegers / Name and address of depositor / Nom et adresse du déposant :</p>
<p>Verzicht auf die Verpflichtung des Antragstellers nach Regel 28(3) in gesondertem Schriftstück / Waiver of the right to an undertaking from the requester pursuant to Rule 28(3) attached</p>	<p>29</p>	<p><input type="checkbox"/> Renonciation, sur document distinct, à l'engagement du requérant au titre de la règle 28(3)</p>
<p>Gemäß Regel 28(4) wird hiermit mitgeteilt, daß der Zugang zu dem in den Feldern 26 und 27 genannten biologischen Material nur durch Herausgabe einer Probe an einen Sachverständigen hergestellt wird / It is hereby declared under Rule 28(4) that the availability of the biological material referred to in Sections 26 and 27 shall be effected only by the issue of a sample to an expert</p> <p style="text-align: right;">BIOM 3</p>	<p>30</p>	<p><input type="checkbox"/> Conformément à la règle 28(4) il est déclaré par la présente que l'accessibilité à la matière biologique mentionnée aux rubriques 26 et 27 ne peut réalisée que par la remise d'un échantillon à un expert</p>
<p>Nucleotid- und Aminosäuresequenzen / Nucleotide and amino acid sequences / Séquences de nucléotides et d'acides aminés</p> <p>Die Beschreibung enthält ein Sequenzprotokoll nach Regel 27a(1) / The description contains a sequence listing in accordance with Rule 27a(1) / La description contient une liste de séquences selon la règle 27bis(1)</p> <p>Der vorgeschriebene Datenträger ist beigefügt / The prescribed data carrier is enclosed / Le support de données prescrit est joint</p> <p>Es wird hiermit erklärt, daß die auf dem Datenträger gespeicherte Information mit dem schriftlichen Sequenzprotokoll übereinstimmt (Regel 27a(2)) / It is hereby stated that the information recorded on the data carrier is identical to the written sequence listing (Rule 27a(2)) / Il est déclaré par la présente que l'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite (règle 27bis(2))</p> <p style="text-align: right;">SEQL 1</p>	<p>31</p> <p><input checked="" type="checkbox"/></p> <p><input checked="" type="checkbox"/></p> <p><input checked="" type="checkbox"/></p>	
<p>Benennung der Vertragsstaaten und Erklärungen hierzu</p> <p>1. Hiermit werden sämtliche Vertragsstaaten des EPÜ benannt, die diesem bei Einreichung dieser Anmeldung angehören*.</p> <p>Mit der Zahlung des siebenfachen Betrags einer Benennungsgebühr gelten die Benennungsgebühren für alle Vertragsstaaten als entrichtet (Art. 2 Nr. 3 GebO).</p> <p>2. Es ist derzeit beabsichtigt, weniger als sieben Benennungsgebühren für folgende Vertragsstaaten zu entrichten (bitte Ländercodes und Vertragsstaaten angeben*):</p> <p>(1) <input type="text"/></p> <p>(2) <input type="text"/></p> <p>(3) <input type="text"/></p>	<p>32</p> <p><input checked="" type="checkbox"/></p> <p><input type="checkbox"/></p> <p>(4) <input type="text"/></p> <p>(5) <input type="text"/></p> <p>(6) <input type="text"/></p>	<p>Désignation d'Etats contractants et déclarations à ce propos</p> <p>1. Sont désignés tous les Etats qui sont des Etats contractants de la CBE à la date du dépôt de la présente demande*.</p> <p>Les taxes de désignation sont réputées acquittées pour tous les Etats contractants dès lors qu'un montant correspondant à sept fois la taxe de désignation a été acquitté (art. 2, point 3 du RRT).</p> <p>2. Il est actuellement envisagé de payer moins de sept taxes de désignation pour les Etats contractants suivants (prière d'indiquer codes de pays et Etats contractants*):</p>
<p>Es wird beantrag, für die unter Nr. 2 nicht aufgeführten Vertragsstaaten von der Zustellung von Mitteilungen nach Regel 85a(1) und Regel 69(1) abzusehen.</p> <p>3. Wird ein automatischer Abbuchungsauftrag erteilt (Feld 43), so wird das EPA beauftrag, bei Ablauf der Grundfrist nach Artikel 79(2) den siebenfachen Betrag einer Benennungsgebühr abzubuchen. Ist eine Erklärung unter Nr. 2 abgegeben worden, so sollen die Benennungsgebühren nur für die dort angegebenen Vertragsstaaten abgebucht werden, sofern dem EPA nicht bis zum Ablauf der Grundfrist ein anderslautender Auftrag zugeht.</p>	<p>No communications under Rules 85a(1) or 69(1) need be notified in respect of the contracting states not indicated under No. 2.</p> <p>3. If an automatic debit order has been issued (Section 43), the EPO is authorised, on expiry of the basic period under Article 79(2), to debit seven times the amount of the designation fee. If any states are indicated under No. 2, the EPO shall debit designation fees only for those states, unless it is instructed to do otherwise before expiry of the basic period.</p>	<p>Prière de ne pas procéder à la signification des notifications prévues par les règles 85bis(1) et 69(1) pour les Etats contractants n'ayant pas été mentionnés au n° 2.</p> <p>3. Si un ordre de prélèvement automatique est donné (rubrique 43), il est demandé à l'OEB de prélever, à l'expiration du délai normal visé à l'article 79(2), un montant correspondant à sept fois la taxe de désignation. Si une déclaration a été faite au n° 2, les taxes de désignation ne sont prélevées que pour les Etats contractants qui y sont indiqués, sauf instruction contraire reçue par l'OEB avant l'expiration du délai normal.</p>

* Stand bei Drucklegung: 19 Vertragsstaaten, und zwar: / Status when this form was printed: 19 contracting states, namely / Situation à la date d'impression: 19 Etats contractants, à savoir: **AT** Österreich / Austria, **BE** Belgien / Belgium, **CH/LI** Schweiz und Liechtenstein / Switzerland and Liechtenstein, **CY** Zypern / Cyprus / Chypre, **DE** Deutschland / Germany, **FR** Frankreich / France, **DK** Dänemark / Denmark, **ES** Spanien / Spain / Espagne, **FI** Finnland / Finland, **GR** Griechenland / Greece / Grèce, **IE** Irland / Ireland, **IT** Italien / Italy / Italie, **LU** Luxemburg / Luxembourg, **MC** Monaco / Monaco, **NL** Niederlande / Netherlands / Pays-Bas, **PT** Portugal / Portugal, **SE** Schweden / Sweden / Suède

<p>Verschiedene Anmelder für verschiedene Vertragsstaaten / Different applicants for different contracting states / Différents demandeurs pour différents Etats contractants</p> <p>APPR 02 # # </p>	<p>33 Name(n) des (der) Anmelder(s) und benannte Vertragsstaaten / Name(s) of applicant(s) and designated contracting states / Nom(s) du (des) demandeur(s) et des Etats contractants désignés</p>												
<p>Erstreckung des europäischen Patents</p> <p>Diese Anmeldung gilt als Antrag, die europäische Patentanmeldung und das darauf erteilte europäische Patent auf alle Nicht-Vertragsstaaten des EPÜ zu erstrecken, mit denen am Tag ihrer Einreichung „Erstreckungsabkommen“ bestehen (derzeit: Albanien, Litauen, Lettland, Rumänien, Slowenien, ehemalige jugoslawische Republik Mazedonien). Die Erstreckung wird jedoch nur wirksam, wenn die vorgeschriebene Erstreckungsgebühr entrichtet wird.</p>	<p>Extension of the European patent</p> <p>This application is deemed to be a request to extend the European patent application and the European patent granted in respect of it to all non-contracting states to the EPC with which "extension agreements" exist on the date on which the application is filed (Present situation: Albania, Lithuania, Latvia, Romania, Slovenia, former Yugoslav Republic of Macedonia). However, the extension only takes effect if the prescribed extension fee is paid.</p>												
<p>Es ist derzeit beabsichtigt, die Erstreckungsgebühr für die nachfolgend angekreuzten Staaten zu entrichten: / It is currently intended to pay the extension fee for the states marked below with a cross: / Il est actuellement envisagé de payer la taxe d'extension pour les Etats dont le nom est coché ci-après :</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%;">Albanien / Albania / Albanie</td> <td style="width: 20%; text-align: center;">AL</td> </tr> <tr> <td>Litauen / Lithuania / Lituanie</td> <td style="text-align: center;">LT</td> </tr> <tr> <td>Lettland / Latvia / Lettonie</td> <td style="text-align: center;">LV</td> </tr> <tr> <td>Rumänien / Romania / Roumanie</td> <td style="text-align: center;">RO</td> </tr> <tr> <td>Slowenien / Slovenia / Slovénie</td> <td style="text-align: center;">SI</td> </tr> <tr> <td>Ehemalige jugoslawische Republik Mazedonien / Former Yugoslav Republic of Macedonia / Ex-République yougoslave de Macédoine</td> <td style="text-align: center;">MK</td> </tr> </table>	Albanien / Albania / Albanie	AL	Litauen / Lithuania / Lituanie	LT	Lettland / Latvia / Lettonie	LV	Rumänien / Romania / Roumanie	RO	Slowenien / Slovenia / Slovénie	SI	Ehemalige jugoslawische Republik Mazedonien / Former Yugoslav Republic of Macedonia / Ex-République yougoslave de Macédoine	MK	<p>34 <input checked="" type="checkbox"/> Extension des effets du brevet européen</p> <p>La présente demande est réputée constituer une requête en extension des effets de la demande de brevet européen et du brevet européen délivré sur la base de cette demande à tous les Etats non parties à la CBE avec lesquels il existe un «accord d'extension» à la date du dépôt de la demande (Situation actuelle : Albanie, Lituanie, Lettonie, Roumanie, Slovénie, ex-République yougoslave de Macédoine). Toutefois, l'extension ne produit ses effets que s'il est acquitté la taxe d'extension prescrite.</p>
Albanien / Albania / Albanie	AL												
Litauen / Lithuania / Lituanie	LT												
Lettland / Latvia / Lettonie	LV												
Rumänien / Romania / Roumanie	RO												
Slowenien / Slovenia / Slovénie	SI												
Ehemalige jugoslawische Republik Mazedonien / Former Yugoslav Republic of Macedonia / Ex-République yougoslave de Macédoine	MK												
<p>(Platz für Staaten, mit denen nach Drucklegung dieses Formblatts „Erstreckungsabkommen“ in Kraft treten) / (Space for states with which "extension agreements" enter into force after this form has been printed) / (Prévu pour des Etats à l'égard desquels des «accords d'extension» entreraient en vigueur après l'impression du présent formulaire)</p>	<p>35 <input type="checkbox"/> Nummer der früheren Anmeldung No. of earlier application Numéro de la demande initiale</p>												
<p>Die Anmeldung ist eine Teilanmeldung / The application is a divisional application / La présente demande constitue une demande divisionnaire</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%; border: 1px solid black; padding: 2px;">DFIL 9</td> <td style="width: 70%; border: 1px solid black; padding: 2px;"># </td> </tr> <tr> <td style="border: 1px solid black; padding: 2px;">PANR</td> <td style="border: 1px solid black; padding: 2px;"># </td> </tr> </table>	DFIL 9	# 	PANR	# 	<p>36 <input type="checkbox"/> Nummer der früheren Anmeldung No. of earlier application Numéro de la demande initiale</p>								
DFIL 9	# 												
PANR	# 												
<p>Es handelt sich um eine Anmeldung nach Artikel 61(1)(b) / The application is an Article 61(1)(b) application / La présente demande constitue une demande selon l'article 61(1)(b)</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%; border: 1px solid black; padding: 2px;">DFIL 9</td> <td style="width: 70%; border: 1px solid black; padding: 2px;"># </td> </tr> <tr> <td style="border: 1px solid black; padding: 2px;">EANR</td> <td style="border: 1px solid black; padding: 2px;"># </td> </tr> </table>	DFIL 9	# 	EANR	# 	<p>37 <input type="checkbox"/> Zahl der Patentansprüche Number of claims Nombre de revendications</p>								
DFIL 9	# 												
EANR	# 												
<p>Patentansprüche / Claims / Revendications</p> <p style="text-align: right; border: 1px solid black; padding: 2px; width: fit-content; margin: 0 auto;">CLMS</p>	<p>39 <input type="checkbox"/> Fig. 1 Nummer / Number / Numéro</p>												
<p>Zur Veröffentlichung mit der Zusammenfassung wird vorgeschlagen Abbildung Nr. / It is proposed that the abstract be published together with figure No. / Il est proposé de publier avec l'abrége la figure n°</p> <p style="text-align: right; border: 1px solid black; padding: 2px; width: fit-content; margin: 0 auto;">DRAW 2</p>	<p>39 <input type="checkbox"/> Fig. 1 Nummer / Number / Numéro</p>												

Zusätzliche Abschrift(en) der im europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt / Additional copy(ies) of the documents cited in the European search report is (are) requested / Prière de fournir une (des) copie(s) supplémentaire(s) des documents cités dans le rapport de recherche européenne <div style="border: 1px solid black; padding: 2px; display: inline-block;">ASOC</div>	40 <div style="border: 1px solid black; padding: 2px; display: inline-block;">1</div> Anzahl der zusätzlichen Sätze von Abschriften / Number of additional sets of copies / Nombre de jeux supplémentaires de copies
Es wird die Rückerstattung der Recherchegebühr gemäß Art. 10 GebO beantragt / Refund of the search fee is requested pursuant to Article 10 of the Rules relating to Fees / Le remboursement de la taxe de recherche est demandé en vertu de l'article 10 du règlement relatif aux taxes Eine Kopie des Recherchenberichts ist beigelegt / A copy of the search report is attached / Une copie du rapport de recherche est jointe	41 <div style="border: 1px solid black; width: 20px; height: 15px; display: inline-block;"></div> 42 <div style="border: 1px solid black; width: 20px; height: 15px; display: inline-block;"></div>
<div style="display: flex;"> <div style="flex: 1;"> Automatischer Abbuchungsauftrag <i>(nur möglich für Inhaber von beim EPA geführten laufenden Konten)</i> Das EPA wird hiermit beauftragt, fällig werdende Gebühren und Auslagen, nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren vom nebenstehenden laufenden Konto abzubuchen. In Bezug auf die Benennungsgebühren wird auf Feld 32.3 verwiesen. Das EPA wird ferner beauftragt, die Erststreckungsgebühren für jeden in Feld 34 angekreuzten »Erststreckungsstaat« bei Ablauf der Grundfrist zu ihrer Zahlung abzubuchen, sofern ihm nicht bis dahin ein anderslautender Auftrag zugeht. </div> <div style="flex: 1;"> Automatic debit order <i>(for EPO deposit account holders only)</i> The EPO is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account opposite any fees and costs falling due. With regard to designation fees reference is made to Section 32.3. The EPO is also authorised, on expiry of the basic period for its payment, to debit the extension fee for each of the "extension states" marked with a cross in Section 34, unless it is instructed to do otherwise before expiry of this period. </div> </div> <div style="text-align: center; margin-top: 10px;"> Für automatischen Abbuchungsauftrag: For automatic debit order: Pour l'ordre de prélèvement automatique: </div> <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-top: 5px;">DECA</div>	43 Ordre de prélèvement automatique <i>(possibilité offerte uniquement aux titulaires de comptes courants ouverts auprès de l'OEB)</i> Par la présente, il est demandé à l'OEB de prélever du compte courant ci-dessous les taxes et frais venant à échéance, conformément à la réglementation relative à la procédure de prélèvement automatique. Pour les taxes de désignation , se reporter à la rubrique 32.3. Il est en outre demandé à l'OEB de prélever, à l'expiration du délai normal prévu pour leur paiement, les taxes d'extension pour chaque «Etat autorisant l'extension» coché à la rubrique 34, sauf instruction contraire reçue avant l'expiration de ce délai. <div style="display: flex; justify-content: space-between;"> <div> Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant </div> <div> Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte </div> </div> <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-top: 5px;">2811.0010</div> <div style="margin-left: 20px;">NESTEC S. A.</div>
Eventuelle Rückzahlungen auf das nebenstehende beim EPA geführte laufende Konto / Reimbursement , if any, to EPO deposit account opposite / Remboursements éventuels à effectuer sur le compte courant ci-contre ouvert auprès de l'OEB <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-top: 5px;">DEPA</div>	44 <div style="display: flex; justify-content: space-between;"> <div> Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant </div> <div> Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte </div> </div> <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-top: 5px;">2811.0010</div> <div style="margin-left: 20px;">NESTEC S. A.</div>
Die vorgeschriebene Liste über die diesem Antrag beigelegten Unterlagen ergibt sich aus der vorbereiteten Empfangsbescheinigung (Seite 6 dieses Antrages) The prescribed list of documents enclosed with this request is shown on the prepared receipt (page 6 of this request)	45 La liste prescrite des documents joints à cette requête figure sur le récépissé préétabli (page 6 de la présente requête)
Unterschrift(en) des (der) Anmelders(s) oder Vertreter(s) / Signature(s) of applicant(s) or representative(s) / Signature(s) du (des) demandeur(s) ou du (des) mandataire(s) Ort / Place / Lieu <u>Munich</u> Datum / Date <u>April 25, 2001</u>	46 Für Angestellte nach Artikel 133(3) Satz 1 mit allgemeiner Vollmacht / For employees under Article 133(3), 1st sentence, having a general authorisation / Pour les employés mentionnés à l'article 133(3), 1 ^{re} phrase, munis d'un pouvoir général Nr. / No. / n° :


Dr. Alexander Straus - European Patent Attorney -

Name des (der) Unterzeichneten bitte in Druckschrift wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft in Druckschrift angeben. / Please print name under signature. In the case of legal persons, the position of the signatory within the company should also be printed. / Le ou les noms des signataires doivent être indiqués en caractères d'imprimerie. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires doit être indiquée en caractères d'imprimerie.

Empfangsbescheinigung / Receipt for documents / Récépissé de documents 6

(Liste der diesem Antrag beigelegten Unterlagen)

(Checklist of enclosed documents)

(Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents désignés ci-dessous

Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA). **Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen.** / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA). **Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office.** / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé de documents, ce récépissé est réputé être la notification visée à la règle 24(4) (cf. rubrique RENA). **Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.**

BECKER KURIG STRAUS
BAVARIASTRASSE 7 · 80336 MÜNCHEN

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Datum / Date



Europäisches Patentamt
European Patent Office
Office européen des brevets
D-80295 München
W. Kiendl

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Anmeldenummer / Application No. / N° de la demande		01110251.4	
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	25. 04. 01	
Zeichen des Anmelders/Vertreters / Applicant's/ Representative's ref. / Référence du demandeur ou du mandataire	AREF	E 80273 EP	
Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: / Seulement après le dépôt de la demande auprès d'un service national:			
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA		

A. Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité	47	Stückzahl / Number of copies / Nombre d'exemplaires	Blattzahl* eines Stücks / Number of sheets* in each copy / Nombre de feuilles* par exemplaire	Gesamtzahl der Abbildungen* / Total number of figures* / Nombre total de figures*
1. Beschreibung (ohne Sequenzprotokollteil) / Description (excluding sequence listing part) / Description (sauf partie réservée au listage des séquences)		3	10	
2. Patentansprüche / Claim(s) / Revendication(s)		3	1	
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5. Zusammenfassung / Abstract / Abrégé		3	1	
6. Übersetzung der Anmeldungsunterlagen / Translation of the application documents / Traduction des pièces de la demande				
7. Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité				
8. Übersetzung des (der) Prioritätsbelegs(belege) / Translation of priority document(s) / Traduction du (des) document(s) de priorité				
B. Der Anmeldung in der eingereichten Fassung liegen folgende Unterlagen bei: / This application as filed is accompanied by the items below: / A la présente demande sont annexées les pièces suivantes:	48			
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2. Allgemeine Vollmacht / General authorisation / Pouvoir général	<input type="checkbox"/>			
3. Erfindernennung / Designation of inventor / Désignation de l'inventeur	<input type="checkbox"/>			
4. Früherer Recherchenbericht / Earlier search report / Rapport de recherche antérieure	<input type="checkbox"/>			
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8. Zusatzblatt / Additional sheet / Feuille additionnelle	<input type="checkbox"/>			
9. Sonstige Unterlagen (bitte hier spezifizieren) / Other documents (please specify here) / Autres documents (veuillez préciser)	<input type="checkbox"/>			
C. Kopien dieser Empfangsbescheinigung / Copies of this receipt for documents / Copies du présent récépissé de documents	49			
	<input checked="" type="checkbox"/> 1 dicke			
	2	Anzahl der Kopien / Number of copies / Nombre de copies		

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File Number : 01110251 . 4

Date of Receipt : 25. 04. 01

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To : Keasberry Peter
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<120> Cocoa polypeptides and their use in the production of
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**Cocoa polypeptides and their use in the production of
cocoa and chocolate flavour**

5

10

The present invention pertains to novel cocoa polypeptides having a molecular weight of about 10 and 14 kDa and being derived from a 69 KDa precursor. In particular, the present invention relates to the production of said polypeptides via recombinant means and the use of said polypeptides or fragments thereof for the production of cocoa/chocolate flavour.

15

During the processing of cocoa beans the generation of the typical cocoa flavour requires two steps – a fermentation step, which includes air-drying of the fermented material, and a roasting step.

20

During fermentation the pulp surrounding the beans is degraded by micro-organisms with the sugars contained in the pulp being largely transformed to acids. In the course of the fermentative process these acids slowly diffuse into the beans eventually causing an acidification of the cellular material. During fermentation also peptides exhibiting differing sizes and a high level of hydrophobic free amino acids are generated, which action is mainly due to the activity of specific proteinases. This specific mixture of peptides and hydrophobic amino acids is deemed to represent cocoa-specific flavour precursors.

25

Research has focused on the different proteolytic enzymes involved in these reactions. In fact a number of different types of enzymes, such as an aspartic endoproteinase, a cysteine endoproteinase or a carboxypeptidase has been found to participate in these degradative reactions leading to the formation of the cocoa flavour peptide/amino acid precursor pool.

30

During the second step of cocoa flavour production – the roasting step – the oligopeptides and amino acids generated at the stage of fermentation are subjected to a Maillard reaction

with reducing sugars present in the mixture eventually yielding substances responsible for the cocoa flavour as such.

In the art there have been attempts to artificially produce cocoa flavour, such as e.g. by
5 subjecting acetone dried powder prepared from unfermented ripe cocoa beans to autolysis at
a pH of 5.2 followed by roasting in the presence of reducing sugars. It was conceived that
under these conditions preferentially free hydrophobic amino acids and hydrophilic peptides
should be generated and the peptide pattern thus obtained was found to be similar to that of
extracts from fermented cocoa beans. An analysis of free amino acids revealed that Leu, Ala,
10 Phe and Val were the predominant amino acids liberated in fermented beans or autolysis
(Voigt et al., Food Chem. 49 (1994), 173-180). In contrast to these findings no cocoa-specific
flavour could be detected when the above powder was subjected to autolysis at a pH of 3.5.
Only few free amino acids were found to be released but a large number of hydrophobic
peptides were formed.

15 Also, with a synthetic mixture of free amino acids alone whose composition resembled that
of the spectrum found in fermented beans, cocoa flavour could not be detected after roasting,
indicating that both the peptides and the amino acids are important for this purpose (Voigt et
al., Food Chem. 49 (1994), 173-180).

20 So far only little attention has been paid to the protein pool from which the peptide/amino
acid flavour precursor pool is generated, since cocoa proteins are difficult to isolate and/or to
investigate. One of the major reasons for this resides in that cocoa seeds contain a high
amount of polyphenols and fat, the depletion of which made the use of lipophilic organic
25 liquids, such as acetone, necessary. Yet, the use of such liquids also contributes to a removal
of flavour active substances or precursors thereof, which are of lipophilic nature. Another
drawback, when trying to elucidate the protein pool of cocoa beans, resides in the poor
solubilisation of proteins purified with acetone, which results in a poor recovery of the total
proteins.

30 So far four major proteins with an apparent molecular weight of 14.5, 21, 31 and 47 kDa,

could be identified in cocoa beans before fermentation, which are deemed to be the main source for the peptide/amino acid pool eventually accounting for cocoa flavour.

Since so far no further substantial information about the cocoa protein/peptide pool is presently available it is an object of the present invention to further elucidate said pool in more detail and eventually provide means for improving the preparation of cocoa flavour.

The above object has been solved by two peptides having a molecular weight of about 10 and 14 kDa, respectively, and being derived from the 69 kDa precursor protein previously described by Spencer et al., (1998) US5770433; Spencer and Hodge, (1991) WO/19801, and Spencer and Hodge, *Planta* 186 (1992), 567-576. The sequence of said peptides is as identified by SEQ. ID. No. 1 and 2, respectively.

In the figures,

Fig. 1 shows photograph of a two-dimensional SDS-PAGE proteins isolated from unfermented cocoa beans;

Fig. 2a shows the result of a LC_ESI-MS analysis of a GndHCL extract of unfermented cocoa beans.

During the studies leading to the invention the present inventors have designed novel methods for an improved isolation of cocoa proteins by using denaturing agents, such as e.g. SDS (1%), urea or GndHCl, which resulted in an about 3-fold increase in solubility of proteins as compared to conventional methods. In particular, the use of 6 M GndHCl provided good results. GndHCl showed up to be easily removable by RP-HPLC and no reaction with the proteins occurred. Moreover, it could also be shown that even crude bean powder, after subjection to a treatment with a solubilization buffer including a denaturing agent could be successfully analysed, which made no special care necessary to remove polyphenols.

During the above-described studies directed to provide a better total recovery of cocoa proteins, the inventors run a crude cocoa bean powder on a two-dimension SDS-PAGE gel, whereby a cluster of several polypeptides exhibiting a molecular weight of about 9 - 16 kDa, could be detected. The polypeptides contained in the acidic cluster have been further isolated by making use of RP-HPLC.

Finally two polypeptides could be isolated showing a molecular weight of about 10 and about 14 kDa, which polypeptides have N-terminally been sequenced. The sequences obtained are shown in SEQ ID. No. 3 (10 Kda protein) and SEQ ID. No 4 (14 kDa protein), respectively.

Upon a comparison with known protein sequences it could be shown that these polypeptides are obviously derived from the 69 KDa precursor protein, which is known to give rise to a 31 and 47 Kda protein. Consequently, said 69 KDa protein, upon processing in cocoa beans not only gives rise to the above mentioned 47 and 31 KDa proteins but also to the present 10 and 14 KDa proteins, which, therefore, also represent part of the protein/peptide pool of cocoa beans.

In consequence, in one embodiment the present invention provides for two polypeptides identified by SEQ. ID. No 1 and SEQ. ID. No. 2, which are part of the protein/peptide pool of unfermented cocoa beans.

According to another embodiment the present invention provides fragments of said polypeptides, obtainable by subjecting said polypeptides to an enzymatic degradation with aspartic endoproteinases, cystein endoproteinases and/or carboxypeptidases.

According to yet another embodiment the invention provides for fragments of said polypeptides obtainable by said enzymatic degradation, which are subsequently reacted with reducing sugars.

According to still another embodiment the present invention provides for a recombinant nucleotide encoding said polypeptides. A nucleotide sequence encoding any of the two

polypeptides may be easily derived from the given polypeptide sequence by translating the amino acid according to the genetic code into corresponding triplets. Such a nucleotide sequence may well be expressed in a suitable cell by means well known in the art, such as e.g. in a bacterial cell, e.g. in *E. coli*, or in yeast, insect cells, mammalian or plant cells.

5

To this end, a nucleotide sequence encoding a polypeptide of the present invention is inserted into a suitable vehicle, such as an expression vector, and is incorporated into a cell of choice. With respect to plant cells, the nucleotides encoding the polypeptides of the present invention may also be incorporated into any of the plant cell's chromosome by using e.g. the phenomenon of homologous recombination. In this respect, at least one copy, preferably more than 40 copies of a nucleotide sequence, encoding any of the present polypeptides may be present on the DNA sequence to be inserted into a plants cell's chromosome.

10

When being inserted into the chromosome of a plant cell, said cell may be stimulated to grow to a plant. Consequently, according to another embodiment the present invention also pertains to a plant containing a recombinant nucleotide encoding a polypeptide of the present invention.

15

Furthermore, the invention provides for the use of said polypeptides for the manufacture of cocoa flavour. To this end it is conceived that the present polypeptides may be added to a fermentation mixture of cocoa beans, in order to provide a higher amount of said polypeptides for degradation. The isolated polypeptides and/or their fragments may be added during cocoa and chocolate processing to enhance these flavors. The isolated polypeptides and/or their fragments can be used in a process reaction with reducing sugars, especially monosaccharides e.g. glucose, fructose etc. to generate artificial cocoa or chocolate flavor. Yet, when using cocoa plants, that have been modified by recombinant means and contain a high number of copies of nucleotide sequences encoding the polypeptides of the present invention, said plants will inherently contain a higher concentration of said polypeptides and eventually will result in the production of a stronger cocoa flavour after the processing.

20

25

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The following examples illustrate the invention in a more detailed manner. It is, however,

understood that the present invention is not limited to the examples but is rather embraced by the scope of the appended claims.

Example 1

5 **Separation of proteins**

Preparation of crude cocoa bean powder

Unfermented cocoa beans were obtained from Ivory Coast and unless stated otherwise all studies were carried out using West African Amelonado cocoa beans. Dried cocoa beans
10 were passed through a bean crusher, followed by a winnower to remove shells. The nibs were kept in a brown bottle at -20°C . Cocoa nibs were milled for few seconds in an universal mill. The nib powder was passed through 0.8-mm sieve and kept at 4°C .

Two-dimensional SDS-PAGE electrophoretic analysis of unfermented cocoa bean

15 Crude (unfermented) cocoa bean powder (100 mg) was dissolved in 1 ml of solubilization buffer [8 M urea, 3 % (w/v) CHAPS, 2.8 % (v/v) carrier ampholytes (ampholine pH range 4-6.5, 5-8, and 3-10 (2:4:1) and 10 mM DTT (dithiothreitol)]. The clear supernatant was subjected to first dimension of separation on an immobilized pH-gradient (IPG) from 4-7, and second dimension on a 10 % T SDS-PAGE gel. Proteins were visualized by silver
20 staining.

The resulting electrophoretic profile of proteins in a typical unfermented cocoa bean on a two-dimensional SDS-PAGE is shown in Fig.1. The 47, 31 and 21 kDa proteins were represented by several subforms and in addition two distinct clusters (acidic and basic)
25 clearly identified in a molecular weight range of about 9-16 kDa. All of the protein spots could be shown to gradually disappear upon fermentation of beans.

A Tricine-SDS-PAGE of unfermented cocoa bean genotypes showed up that said clusters in the molecular weight region 9-16 kDa were present in all of the 21 different genotypes
30 representing three cocoa groups, namely Criollo, Forastero and Trinitario.

The acidic cluster has been selected for further investigation.

Example 2

Isolation of proteins having a molecular weight of about 9 - 16 KDa

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Preparation of an GndHCl extract of CAP

Cocoa nib powder (10g) (supra) was suspended in 200 ml 80 % (v/v) aqueous acetone and stirred for 1 hr at 4 °C. The resulting suspension was centrifuged at 15,000 rpm for 15 min at 4 °C. The residue was extracted 5-times with 200 ml 80 % (v/v) aqueous acetone followed by 3-times washing with 100 % acetone. The resulting acetone powder was dried under reduced pressure.

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Subsequently a GndHCl extract and a pyridine-ethylated GndHCl extract of CAP from unfermented cocoa beans was prepared. 1 g CAP was suspended with 10 ml GndHCl buffer (100 mM ammonium phosphate, 66.7 mM potassium hydroxide, 3 mM EDTA and 6 M GndHCl) and sonicated for 1 min. The suspension was cooled on ice for 15-30 min and centrifuged at 15000 rpm at 4 °C for 15 min. The clear supernatant was carefully removed. In order to obtain a pyridine-ethylated GndHCl extract, said CAP extract (2 ml) was sparged with argon and mixed with 50 µl of reducing solution (0.8 M DTT in 3 M tripotassium phosphate/3 mM EDTA). The solution was kept at room temperature in dark for 60 min. Pyridine-ethylation at cysteine residues of the reduced CAP was carried out by mixing vigorously 40 µl of 4-vinyl pyridine and further incubation for 30 min at room temperature (Lundell and Schreitmüller, Anal. Biochem. 266 (1999) 31-47). The reaction mixture was dialyzed against 500 ml of the extraction buffer for overnight at room temperature. The dialyzed sample was centrifuged and the clear supernatant passed through 0.22 µm filter disc and kept at 4 °C until analyzation.

LC-ESI-MS analysis of the reduced and pyridylethylated GndHCl-extract

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A LC-ESI-MS analysis of the reduced and pyridylethylated extract was performed, as may be seen from FIG. 2. To this end, reduced and pyridylethylated GndHCl extracts of CAP were injected onto reverse phase HPLC columns [Bio-Rad HRLC series 800 system; columns C4

and C8 from Aquapore RP butyl (7 μ m, 4.6 x 220 mm), Aquapore RP 300 (7 μ m, 4.6 x 220 mm), Perkin-Elmer; Spherisorb 80-5C8 (220 x 4 mm); Marchery Nagel and Vydac protein C4 (4.6 x 220 mm)) pre-equilibrated with solvent A (0.1 % v/v TFA in water) and eluted with a linear gradient of increasing concentration of solvent B (80 % v/v acetonitrile and 0.1 % v/v TFA): 0-15 % B in 5 min, 15-27 % B in 40 min, 27-35 % B in 2 min, isocratic at 35 % B for 3 min, 35-43 % B in 25 min, 43-56 % B in 50 min, 56-70 % B in 5 min, 70-100 % B in 10 min and isocratic at 100 % B for 5 min].

Fractions containing proteins were found to elute at a retention time of about 41, 52, 68, 78 and 87 min, were of about M_r 10,425 (marked as CSP10), 9,010 (marked as CSP9), 20,540 (marked as CSP22) and 12,500 (marked as CSP12), respectively, as can be seen in Table 1. In the case of proteins eluting at 41 min and 97 min, no molecular mass could be identified.

Table 1

Retention time, min	Sample code	Average M_r	Comments
41.4	CSP14	Not detected	
52.5	CSP10	10,425	
67.7	CSP9	9,010	
78.5	CSP22	20,540	Albumin CSP
86.9	CSP12	12,245	
97.3	CSP67	Not detected	Vicilin type CSP
132.2	CSPAgg	Not detected	

Since the average M_w of the protein designated CSP14 could not be assigned with the above method, the peak fraction was dissolved in 500 μ l of 25 % solvent B (0.05 % (v/v) TFA/80 %, v/v ACN). For SDS-PAGE, a 10 μ l aliquot was dried in speedvac and dissolved in 20 μ l SDS-sample buffer and analyzed on gradient 10-20% T ready Tris-Tricine acrylamide gels using the miniprotean 3 system from Bio-Rad. Protein bands were visualized by staining the gels in the staining solution [0.5 % (w/v) Commassie Brilliant Blue R250 in 30 % (v/v) methanol and 10 % (v/v) acetic acid] for 1 hr followed by destaining [30 % (v/v) methanol plus 10 % (v/v) acetic acid] until bands were visible against the clear background (Graffin, Methods Enzymol. 182 (1990) 425-477). Accordingly it could be observed that CSP 14 corresponds to a protein having a molecular weight of about 14 kDa.

Purification/collection by repetitive RP-HPLC

Subsequently, the cocoa proteins, reduced and pyridine-ethylated were isolated/collected by repetitive injections and automatic fraction pooling and collection from the GndHCl extracts of unfermented CAP, as described above. The pooled fractions of each proteins were dried under reduced pressure and dissolved in 400 µl solvent A and rechromatographed [column Aquapore RP 300 (7 µm, 4.6 x 220 mm), solvent TFA/ACN system; injection volume 400 µl; detection at 215 nm; gradients: 1. (CSP14) and (CSP9): 0-15 % B in 5 min, isocratic at 15 % B for 5 min, 15-35 % B in 60 min, 35-50 % B in 10 min, 50-100 % B in 5 min and isocratic at 100 % B for 5 min; 2. FIG. 7c (CSP12): 0-35 % B in 5 min, isocratic at 15 % B for 5 min, 35-60 % B in 60 min, 60-75 % B in 10 min, 75-100 % B in 10 min and isocratic at 100 % B for 10 min;]. Fractions 1 ml each were automatically collected and those containing the peak fractions for each of CSP14 and CSP10 were pooled, dried and kept at -20 °C until used.

Example 3

Characterization of purified proteins

The purified cocoa seed proteins CSP10 and CSP14 were subjected to N-terminal amino acid sequencing by automated Edman degradation protein sequencer. The initial and repetitive yield of Edman cycle was between 80 to 90 %. The results obtained are shown in table 2 below.

Table 2

Protein	Initial amount, pmol	Initial yield, pmol	Sequence
CSP10	400	120	Arg Arg Glu Gln Glu Glu Glu Ser Glu Glu Glu Thr Phe Gly Glu Phe Xaa Gln Val Xaa Ala Pro Leu Xaa Pro Gly
CSP14	200	100	Gly Arg Lys Gln Tyr Glu Arg Asp Pro Arg

The above listed N-terminal sequence of CSP 10 and 14 has been found to be a part of the 67 kDa vicilin type cocoa storage protein (WO 91/19801, supra). Thus, both of CSP 10 and

14 are so far not identified fragments of the 67 kDa vicilin type cocoa storage protein produced during the normal processing of said protein in cocoa beans. By aligning the 47 and 31 Kda proteins, known to be derived from the 67 KDa vicilin protein, to said protein the remaining sequence for the CSP 10 and 14 could be derived, which yielded the sequences as identified by SEQ. ID. No. 1 and SEQ. ID. No. 2.

A calculation of the molecular weights of the amino acids contained in the polypeptides according to SEQ. ID. No. 1 and 2 confirmed the approximate molecular weights of the resulting polypeptides of about 10 and 14 KDa.

Consequently said peptides also seem to be excised during the normal processing of the 67 KDa protein and represent a part of the protein/polypeptide pool of cacao beans.

Example 4

15 Process Reaction flavors with or without enzymatic treatment of cocoa polypeptides

A reference cocoa reaction flavor was prepared by reacting 0.8 % Leu, 1.45 % Phe, 0.8 % Val, 1.5 % Fructose, 1.5 % water (4 drops of NaOH in 20 ml water) and 94 % propylene glycol at 125 °C for 60 min under reflux. Reaction flavors, prepared with isolated cocoa polypeptide before and after hydrolysis with commercial enzymes or cocoa enzymes were generated by replacing the amino acids with 1 % lyophilized polypeptide fraction. Tasting was performed on a 0.1 % solution in 1 % sucrose. The reaction flavor produced with cocoa hydrolysate were tested and compared against reference.

Claims

1. A cocoa polypeptides identified by SEQ. ID. No. 1.
2. A cocoa polypeptide identified by SEQ. ID. No. 2.
3. A fragment of a polypeptide according to any of the claims 1 or 2, obtainable by enzymatic degradation involving exo-, endo- proteases, aspartic endoproteinase, cysteine endoproteinase, aminopeptidases and/or carboxypeptidase.
4. A fragment according to claim 3, wherein the enzymes are derived from cocoa.
5. A polypeptide or a fragment according to any of the preceding claims, which is further reacted with reducing sugars.
6. A nucleotide sequence encoding a polypeptide according to any of the preceding claims.
7. A vector containing a nucleotide sequence according to claim 6.
8. A cell containing a nucleotide sequence according to claim 6 and/or a vector according to claim 7.
9. A cell according to claim 8, which is a bacterial cell, yeast an insect cell, a mammalian cell or a plant cell.
10. A plant containing a plant cell according to claim 9.
11. Use of a polypeptide or fragments thereof according to any of the preceding claims for the production of cocoa and/or chocolate flavour.

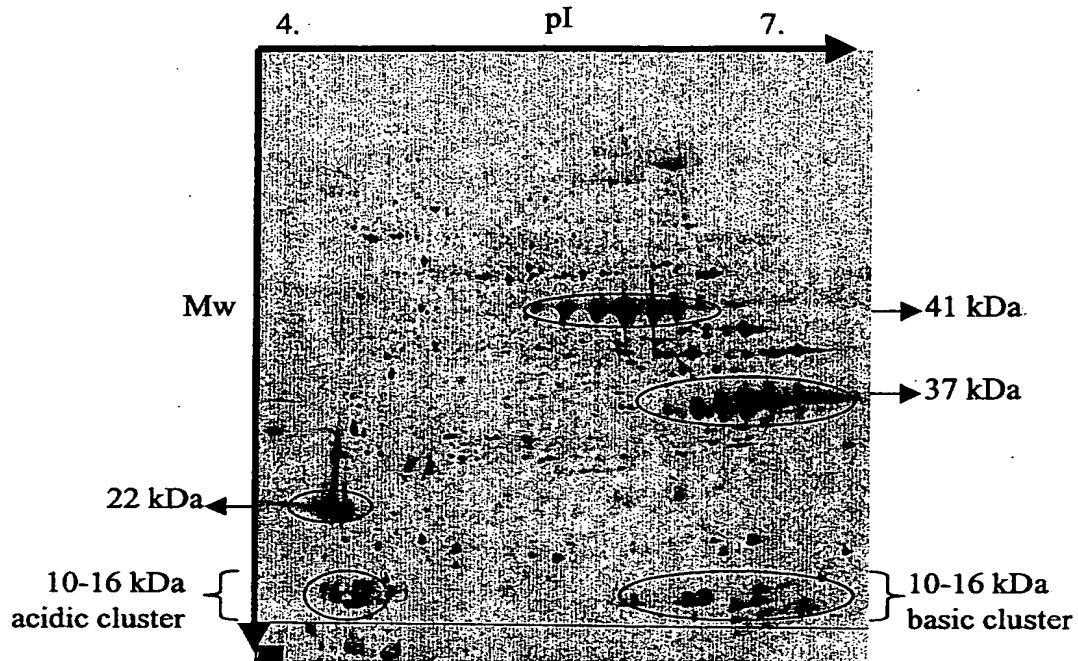


Fig. 1: Two-dimensional SDS-PAGE pattern of unfermented cocoa. Protein spots are visualized by silver staining.

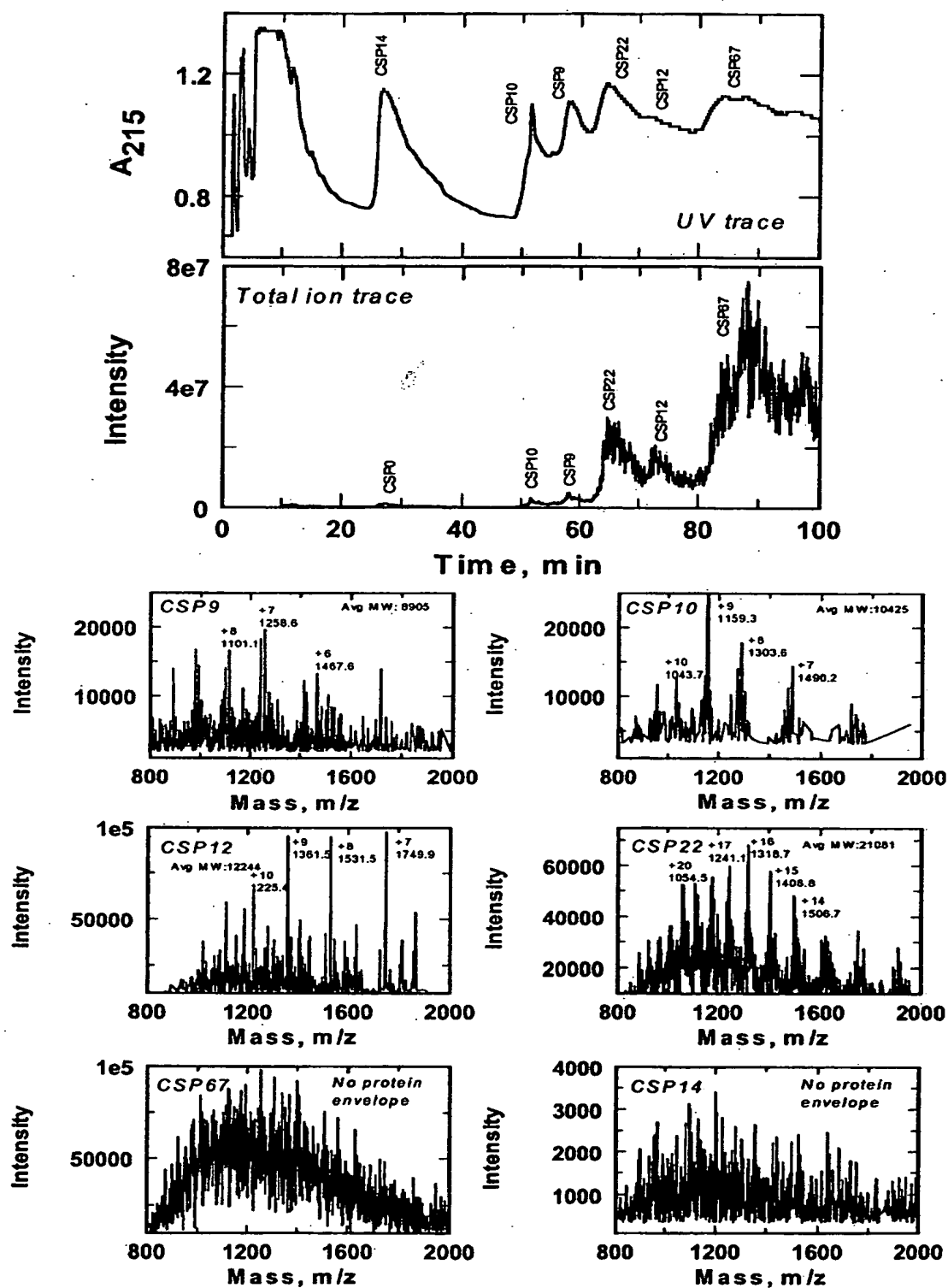


Fig2. LC/MS data for molecular mass determination of cocoa polypeptides

Summary

- 5 The present invention pertains to novel cocoa polypeptides having a molecular weight of about 10 and 14 kDa and being derived from a 69 KDa precursor. In particular, the present invention relates to the production of said polypeptides via recombinant means and the use of said polypeptides or fragments thereof for the production of cocoa/chocolate flavour.

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European Patent Attorneys
European Trademark Attorneys

Dr. Eberhard Becker, Chem.
Dr. Thomas Kurig, Dipl.-Phys.
Dr. Alexander Straus, Dipl.-Chem.

Bavariastraße 7
D-80336 München
Tel.: +49-89-746 303 0
Fax: +49-89-746 303 11
e-mail: info@galileolaw.de

May 11, 2001

European Patent Application EP 01110251.4
Applicant / Owner: Société Des Produits Nestlé S.A.
Our Ref.: 80273 EP (AS/SD)

The following document is herewith filed bilated:

- Designation of inventor.

Dr. Alexander Straus
European Patent Attorney

Bankverbindung:
HypoVereinsbank: Kto. 331 401 110
BLZ 711 200 77
Deutsche Bank: Kto. 951 36 56
BLZ 700 700 10

Büro Berlin
Monumentenstraße 23
D-10965 Berlin
Tel.: +49-30-698 179 10
Fax: +49-30-698 179 11

in Kooperation mit:
HYNELL
PATENTTJÄNST AB
Patron Carls väg 2
SE-683 40 Hagfors/Uddeholm



Erfindernennung
Designation of inventor
Désignation de l'inventeur

(falls Anmelder nicht oder nicht allein der Erfinder ist) /
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N° de la demande ou, s'il n'est pas encore connu, titre de l'invention :

01110251.4

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80273/EP

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In respect of the above European patent application I (we), the undersigned¹

En ce qui concerne la demande de brevet européen susmentionnée, le(s) soussigné(s)¹

Société des Produits Nestlé S.A.

P. O. Box 3 53

1800 Vevey

SWITZERLAND

als Erfinder²:

do hereby designate as inventor(s)²:

désigne(nt) en tant qu'inventeur(s)²:

Sunil Kochhar; Chemin du Grammont; 1073 Savigny; SWITZERLAND

Carl Erik Hansen; Chemin du Pré-d'Yverdon; 1066 Epalinges;
SWITZERLAND

Marcel Alexander Juillerat; Sentier Praz-Dom-Nicod 10;
Vers-chez-les-Blanc; 1000 Lausanne-26; SWITZERLAND

- ☐ Weitere Erfinder sind auf einem gesonderten Blatt angegeben. / Additional inventors indicated on supplementary sheet. /
D'autres inventeurs sont mentionnés sur une feuille supplémentaire.

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Dr. Alexander Straus, Europäischer Patentanwalt

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P.B.5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
☎ +31 70 340 2040
TX 31651 epo nl
FAX +31 70 340 3016

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Kochhar, Sunil
Chemin du Grammont
1073 Savigny
SUISSE

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01110251.4-1221

Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire

Société des Produits Nestlé S.A.

DESIGNATION AS INVENTOR - COMMUNICATION UNDER RULE 17(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the Designation of Inventor and further data mentioned in Art. 128(5) EPC:

DATE OF FILING : 25.04.01

PRIORITY :

TITLE : Cocoa polypeptides and their use in the
production of cocoa and chocolate flavour

DESIGNATED STATES : AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 2):

1/Kochhar, Sunil/Chemin du Grammont/1073 Savigny/CH

1/Hansen, Carl Erik/Chemin du Pré-d'Yverdon/1066 Epalinges/CH

1/Juillerat, Marcel Alexander/Sentier Praz-Dom-Nicod 10 Vers-chez-
les-Blanc/1000 Lausanne-26/CH

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as employer(s).

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P. B. 5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
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TX 31651 epo nl
FAX +31 70 340 3016

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Hansen, Carl Erik
Chemin du Pré-d'Yverdon
1066 Epalinges
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Juillerat, Marcel Alexander
Sentier Praz-Dom-Nicod 10
Vers-chez-les-Blanc
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1/Hansen, Carl Erik/Chemin du Pré-d'Yverdon/1066 Epalinges/CH

1/Juillerat, Marcel Alexander/Sentier Praz-Dom-Nicod 10 Vers-chez-
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FAX +31 70 340 3016

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Département à
La Haye
Division de la
recherche

Becker Kurig Straus
Patentanwälte
Bavariastrasse 7
80336 München
ALLEMAGNE

Datum/Date

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Zeichen/Ref./Réf.

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COMMUNICATION

The European Patent Office herewith transmits as an enclosure the European search report for the above-mentioned European patent application.

If applicable, copies of the documents cited in the European search report are attached.

☒ Additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

The following specifications given by the applicant have been approved by the Search Division:

☒ abstract

☒ title

☐ The abstract was modified by the Search Division and the definitive text is attached to this communication.

The following figure will be published together with the abstract:

1

REFUND OF THE SEARCH FEE

If applicable under Article 10 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.





DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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X	YAVUZ M O ET AL: "Expression of the major bean proteins from Theobroma cacao (cocoa) in the yeasts Hansenula polymorpha and Saccharomyces cerevisiae" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 46, no. 1, 18 April 1996 (1996-04-18), pages 43-54, XP004036811 ISSN: 0168-1656 see Fig. 7 and 8	1-5,7-9, 11	
X	VOIGT J ET AL: "COCOA-SPECIFIC AROMA PRECURSORS ARE GENERATED BY PROTEOLYTIC DIGESTION OF THE VICILIN-LIKE GLOBULIN OF COCOA SEEDS" FOOD CHEMISTRY, ELSEVIER SCIENCE PUBLISHERS LTD, GB, vol. 50, no. 50, 1994, pages 177-184, XP000885588 ISSN: 0308-8146 page 182, left column; Fig. 4	1-5,11	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C07K C12N A01H
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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 30 October 2001	Examiner Holtorf, S
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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	WO 91 06570 A (UNIV MELBOURNE) 16 May 1991 (1991-05-16) * the whole document *		
A	WO 98 27805 A (BOWER NEIL IVAN ;GREEN JODIE LYN (AU); COOPERATIVE RESEARCH CENTRE) 2 July 1998 (1998-07-02) see SEQID7, claims, Fig.4+6; page 14, line 18		
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A	WO 96 38472 A (AARHUS OLIEFABRIK AS ;RASMUSSEN SOEREN (DK); BACH MOGENS (DK)) 5 December 1996 (1996-12-05) * the whole document *		
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 30 October 2001	Examiner Holtorf, S
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EP 01 11 0251

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**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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30-10-2001

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P.B. 5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
☎ +31 70 340 2040
TX 31651 epo nl
FAX +31 70 340 3016

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Patent Office

Receiving
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Office européen
des brevets

Section de
Dépôt

Becker Kurig Straus
Patentanwälte
Bavariastrasse 7
80336 München
ALLEMAGNE

Datum/Date

18/09/02

Zeichen/Ref./Réf.

E 80273 EP

Anmeldung Nr./Application No./Demande n°/Patent Nr./Patent No./Brevet n°.

01110251.4-2405 / 1253200

Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire

Société des Produits Nestlé S.A.

NOTIFICATION OF EUROPEAN PUBLICATION NUMBER AND INFORMATION ON THE APPLICATION OF ARTICLE 67(3) EPC

The Receiving Section hereby informs you that the technical preparations for publication of the above-mentioned European patent application have been completed.

The provisional protection under Art. 67(1) and (2) EPC in the individual Contracting States becomes effective only when the conditions referred to in Art. 67(3) EPC have been fulfilled (for further information, see EPO brochure "National Law relating to the EPC").

This application will be published on 30.10.02 with the European search report. The publication will be mentioned in European Patent Bulletin number 2002/44

The publication number is: 1253200

The title of the invention in the three official languages of the European Patent Office is worded as follows:

Kakao-Polypeptide und ihre Anwendung zur Herstellung von Kakao- und Schokoladenaroma

Cocoa polypeptides and their use in the production of cocoa and chocolate flavour

Polypeptides de cacao et leur utilisation pour la fabrication d'arôme de cacao et de chocolat

In all future communications to the EPO, please quote the application number as indicated above, i.e. including the final four figures (which identify the Directorate responsible for the subsequent procedure). Amendments to a European patent application or European patent must be filed in the language of the proceedings.

REMARK: An issue of the published European patent application will be forwarded to you directly from our printer.

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P.B.5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
☎ (070) 3 40 20 40
FAX (070) 3 40 30 16

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Becker Kurig Straus
Patentanwälte
Bavariastrasse 7
80336 München
ALLEMAGNE



epoline® Customer Services

Tel.: +31 (0)70 340 45 00

Date
05.11.02

Reference E 80273 EP	Application No./Patent No. 01110251.4 - 2405
Applicant/Proprietor Société des Produits Nestlé S.A.	

**Communication pursuant to Rule 50 EPC - reminder of payment of the designation fees
(Art. 79(2) EPC) and of the examination fee (Art. 94(2) EPC)**

The date on which the European Patent Bulletin mentions the publication of the European search report for the above-mentioned European patent application is: 30.10.02.

Your attention is drawn to Article 79(2) and (3) EPC as well as Article 94(2) and (3) EPC according to which within **SIX MONTHS** after the above-mentioned publication date of the search report

- the designation fee(s) must be paid,
- a written request for examination must be filed as well as the examination fee must be paid.
(A written request for examination has been filed already.)

The current rate of the designation fee for each contracting state designated is: EUR 75,00

If the application has been filed on or after 01 July 1999 the payment of seven times the amount of the designation fee is deemed to constitute payment of the designation fees for all contracting states (see OJ EPO 06/1999, 405).

The current rate of the examination fee is: EUR 1430,00

If at least one designation fee and the examination fee are not paid within the period laid down in Article 79(2) or 94(2) EPC, the application shall be deemed to be withdrawn (Arts. 79(3), 94(3) EPC).

Any extension fees are also payable within the above-mentioned period.

**NOTE TO USERS OF THE AUTOMATIC DEBITING PROCEDURE:****1) Designation fees**

If the application has been filed up to 30 June 1999, the designation fees for the contracting states marked under no. 2 of section 32 of the Request for Grant (EPO Form 1001) will be debited on the last day of the period pursuant to Article 79(2) EPC, unless the EPO receives prior instructions to the contrary.

If the application has been filed on or after 01 July 1999, seven times the amount of the designation fee will be debited on the last day of the period pursuant to Article 79(2) EPC. However, if contracting states are marked under no. 2 of section 32 of the Request for Grant (EPO Form 1001), the designation fees only for these contracting states will be debited unless instructions to the contrary have reached the EPO within the basic period for paying the designation fees.

2) Examination fee

Unless the EPO receives prior instructions to the contrary, the examination fee will be debited on the last day of the period for payment.

For further details see the Arrangements for the automatic debiting procedure, Supplement to OJ EPO 02/2002.

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Patent- und Rechtsanwälte
European Patent Attorneys
European Trademark Attorneys

Dr. Eberhard Becker, Chem.
Dr. Thomas Kurig, Dipl.-Phys.
Dr. Alexander Straus, Dipl.-Chem.
Dr. Roman Vuille¹, Dipl.-Chem.
Friedrich von Braun, Rechtsanwalt

Peter Kylin, MSc.²
Magnus Hynell, MSc.²
Annika Björkman, MSc.²
Ivar Andréasson, MSc.²
Magnus Aspeby, MSc.² of counsel
Lars E. Johansson, MSc.² of counsel
Eva Lena Jansson²

Bavariastrasse 7
D-80336 München
Tel.: +49-89-746 303 0
Fax: +49-89-746 303 11
info@galileolaw.de
www.galileolaw.de

EPO - Munich
80
20. Dez. 2002

December 20, 2002
(AS/NL)

European Patent Application EP 01 110 251.4-2405

Applicant / Owner: Société Des Produits Nestlé S.A.

Our Ref.: 80273 EP

This is in response to Communication pursuant to Rule 50 EPC dated #.

We herewith request examination.

Moreover, all contracting states are to be designated.

The examination fee and the designation fees, together amounting EUR 1.955,00, are to be debited from the Nestec S.A. Deposit Account No. 2811.0010.

Dr. Alexander Straus
European Patent Attorney